

## Occurrence of Hematopoietic Neoplasms in Virginia Oysters (*Crassostrea virginica*)



Frierman

E. MICHAEL FRIERMAN

The three predominant pathogens of oysters in Virginia are the haplosporidians *Minchinia costalis* and *M. nelsoni* and the protist *Dermocystidium marinum* (Wood and Andrews, 1962; Haskin, Stauber, and Mackin, 1966; Mackin, Owen, and Collier, 1950). *Minchinia costalis* is known mostly from the high-salinity seaside embayments of Virginia, Maryland, and Delaware from Cape Henry to Cape Henlopen. *Minchinia nelsoni* and *D. marinum* have wide geographic ranges and kill oysters in Chesapeake Bay and Delaware Bay where salinities exceed 15‰. These diseases have inflicted devastating mortalities upon Virginia oyster populations, and large areas of public and rented oyster grounds have been abandoned (Andrews, 1966, 1967, 1968; Andrews and Hewatt, 1957; Andrews and Wood, 1967). Extensive monitoring of oysters held in trays to study diseases (Hewatt and Andrews, 1954) and intensive sampling have provided 70 cases of a rare neoplastic disease similar to that described by Farley (1969a) and Farley and Sparks (1970).

Microscopic studies of the 70 cases found in trayed populations of oysters have not revealed a disease agent. Wide geographic occurrence with vague seasonality reduced the possibility of localized environmental causes.

The disease believed to be neoplasia of the hematopoietic system (Couch, 1969; Farley, 1969a, b; Farley and Sparks, 1970; Pauley, 1969; Wolf, 1969) had the following histologic and cytologic characteristics: 1) Atypical hyaline hemocytes characterized by enlarged nuclei, and unusual abundance of mitotic figures; 2) intensive proliferation of hemocytes; 3) connective tissues invaded and sinuses congested

with hemocytes; and 4) wide range of hemocyte sizes (Figs. 1-4).

The field program at the Virginia Institute of Marine Science for studying diseases of oysters utilized "disease free" native oysters from low-salinity areas of the James River as controls. Oysters were held in iron trays lined with galvanized wire-mesh and suspended off the bottom by 12-inch legs. A zinc bar anode was attached to each tray to extend the life of the liners. Initially, 400 or more oysters were placed in each tray. Trays were distributed in major rivers to monitor monthly *M. nelsoni* activity that occurs in salinities above 15‰. Oysters were counted and cleaned of fouling every 3 or 4 weeks. Samples of 25 live oysters were prepared histologically and examined for diseases and to document their seasonality (Andrews, 1966). Dead oysters, both boxes (no meat) and gapers (meat present) were removed and gaper tissues processed. Trayed groups of known origin and history were monitored regularly from 1 to 10 years, with duration dependent on survival of oysters and the objectives of experiments.

An extensive laboratory oyster-breeding program was initiated at the Virginia Institute of Marine Science in 1964 to produce "superior genetic" strains, i.e., rapid growth, disease resistance. Many experimental lots were field monitored with intensive selection. Two inbred lots, P-58 and P-104, were found with high prevalences of hematopoietic neoplasms. Thirty-one neoplasms were found in 369 oysters from these two groups. In contrast, only 39 hematopoietic neoplasms were recorded from over 51,000 oysters examined from 1964 to 1973 in other progeny lots and native imports.

*E. Michael Frierman is with the Virginia Institute of Marine Science, Gloucester Point, VA 23062. This article is an extended abstract of the full paper "Occurrence of Hematopoietic Neoplasms in Virginia Oysters (Crassostrea virginica)" by E. Michael Frierman and J. D. Andrews, published in the Journal of the National Cancer Institute, Vol. 56, No. 2, February 1976.*

Native and laboratory-bred oysters have shown similar prevalences (0.06 percent in 36,000) from the York River. Hematopoietic neoplasms occurred in 12.5 percent of all groups of oysters from 1964 to 1973. One or more cases appeared in 11.9 percent of 134 native groups and 13.2 percent of 106 laboratory-bred groups. Only lots P-58 and P-104 exhibited relatively high prevalences.

Lot P-104 showed hematopoietic neoplasms that appeared to be associated with mortalities of oysters. A high percentage of gapers with neoplasms occurred during July 1973, and this corresponded to high mortalities and a high percentage of live-oyster infections in the absence of other diseases. Tray lot P-58 had concurrent hematopoietic neoplasms and *M. nelsoni* infections in live samples, and the presence of the two diseases precluded assignment of specific mortalities.

Neoplasms in laboratory-bred oysters have appeared as early as 217 days from spawning and as late as 880 days without clear seasonality. A case was diagnosed in a live oyster about 8 years old. In the susceptible groups, neoplasms appeared at an age of 1 year with severe mortalities. The lot P-58 cases were scattered from August 1969 through August 1970, whereas lot P-104 cases occurred mostly in July 1973 with two cases in November 1973.

A group of 100 James River native oysters was weighed weekly from 1

Figure 1.—Transverse section of *C. virginica* showing massive congestion of sinus by abnormal hyaline hemocytes. Note infiltration of the connective tissue by abnormal hemocytes. 200X.

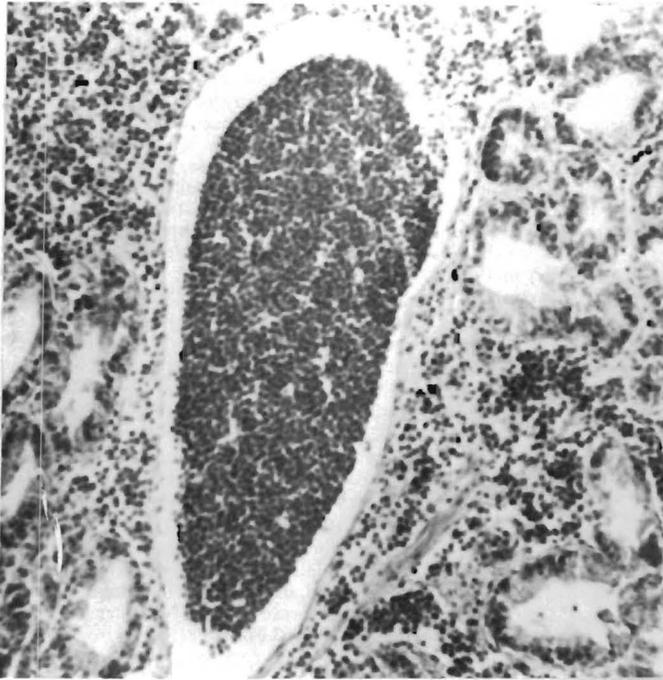
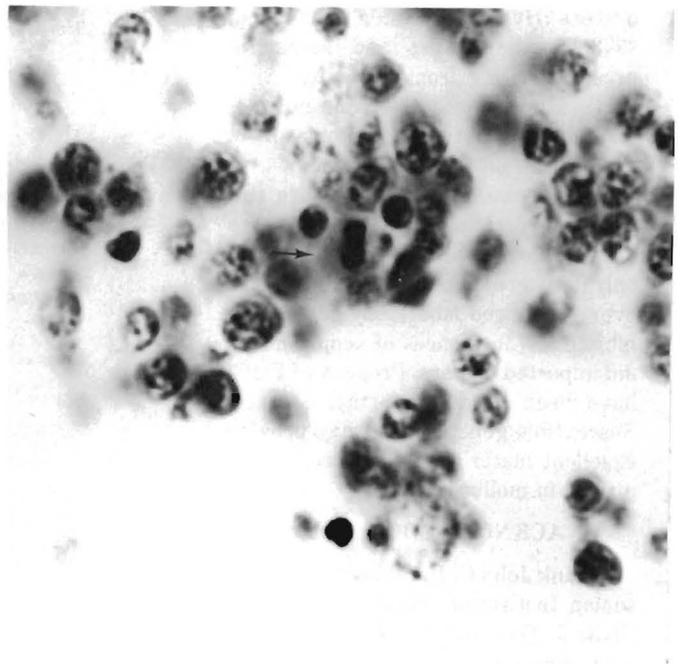
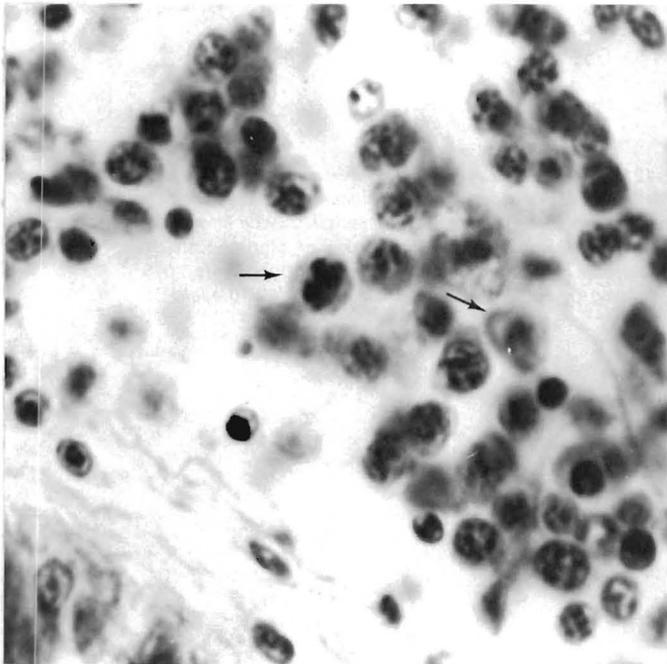
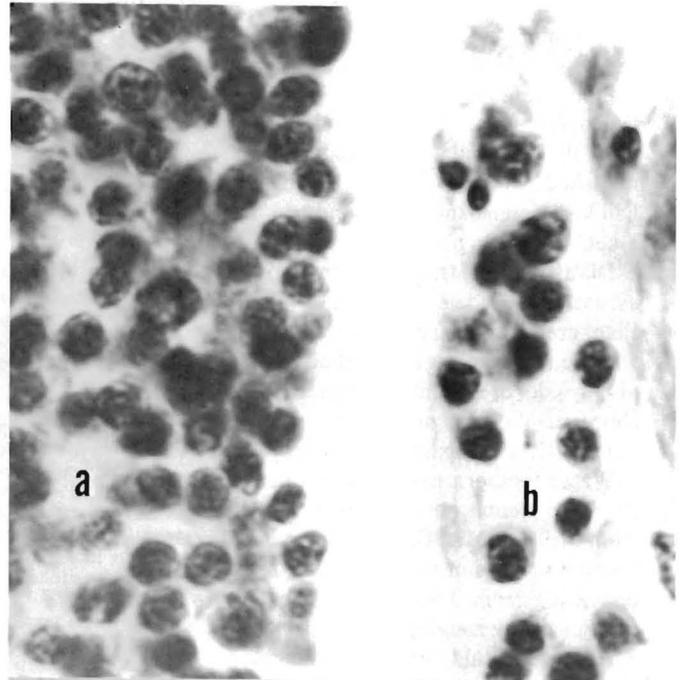


Figure 2.—Abnormal hemocytes in sinus (a) and connective tissue (b). Note enlarged, densely staining nuclei. 1,750X.



Figures 3, 4.—Abnormal hyaline hemocytes. Arrows indicate mitotic figures. 1,750X.

April 1969 by a technique of underwater weighing for growth as indicated by shell deposition (Andrews, 1961). With control of time and source of oyster importation this method permitted detection of sick oysters by lack of weight increase at weekly intervals. One oyster showed normal weight gain until 10 July when shell deposition diminished. On 17 July 1969 the oyster had lost weight and was found to have a hematopoietic neoplasm.

Distribution of the disease in Virginia estuaries included the James, York, Piankatank, and Ware rivers. No cases were found from the high-salinity areas of bayside or seaside of the eastern shore of Virginia or the low-salinity James River "disease free" area.

Water temperatures at which hematopoietic neoplasms occurred ranged from 27.9°C to 1.1°C. Cases have been found 9 months of the year, with most appearing from July through November in salinity ranging from 10 to 22‰.

Heavy metals analysis indicated above normal levels of zinc in trayed oysters (Huggett, Bender, and Slone, 1973; Huggett, Cross, and Bender, in press) resulting from galvanized tray parts. However, at this time no relationship to hematopoietic neoplasms in oysters can be ascribed to zinc.

This disease may appear more frequently in certain genetic combinations of inbred oysters. Higher incidence in only two groups suggests this, since over 100 inbred laboratory groups exhibited low incidences of neoplasms as did imported oysters. Progeny of P-104 have been bred for further studies. Susceptible genetic races may provide excellent materials for studies of neoplasms in mollusks.

#### ACKNOWLEDGMENTS

I thank John C. Harshbarger, Smithsonian Institution, Washington, D.C.; Clyde J. Dawe, National Cancer Institute, Bethesda, Md.; and C. Austin Farley, National Marine Fisheries Service, Oxford, Md., for their assistance in identifying the diseases.

#### LITERATURE CITED

- Andrews, J. D. 1961. Measurement of shell growth in oysters by weighing in water. Proc. Natl. Shellfish. Assoc. 52:1-11.
- \_\_\_\_\_. 1966. Oyster mortality studies in Virginia. V. Epizootiology of MSX, protistan pathogen of oysters. Ecology 47:19-31.

- \_\_\_\_\_. 1967. Interaction of two diseases of oysters in natural waters. Proc. Natl. Shellfish. Assoc. 57:38-49.
- \_\_\_\_\_. 1968. Oyster mortality studies in Virginia. VII. Review of epizootiology and origin of *Minchinia nelsoni*. Proc. Natl. Shellfish. Assoc. 58:23-36.
- Andrews, J. D., and W. G. Hewatt. 1957. Oyster mortality studies in Virginia. II. The fungus disease caused by *Dermocystidium marinum* in oysters of Chesapeake Bay. Ecol. Monogr. 27:1-25.
- Andrews, J. D., and J. L. Wood. 1967. Oyster mortality studies in Virginia. VI. History and distribution of *Minchinia nelsoni*, a pathogen of oysters, in Virginia. Chesapeake Sci. 8:1-13.
- Couch, J. A. 1969. An unusual lesion in the mantle of the American oyster, *Crassostrea virginica*. In Neoplasms and related disorders of invertebrate and lower vertebrate animals. Natl. Cancer Inst. Monogr. 31:557-562.
- Farley, C. A. 1969a. Probable neoplastic disease of the hematopoietic system in oysters, *Crassostrea virginica* and *Crassostrea gigas*. In Neoplasms and related disorders of invertebrate and lower vertebrate animals. Natl. Cancer Inst. Monogr. 31:541-555.
- \_\_\_\_\_. 1969b. Sarcomatoid proliferative disease in a wild population of blue mussels (*Mytilus edulis*). J. Natl. Cancer Inst. 43:509-516.
- Farley, C. A., and A. K. Sparks. 1970. Proliferative diseases of hemocytes, endothelial cells, and connective tissue cells in mollusks. Bibl. Haematol. 36:610-617.
- Haskin, H. H., L. A. Stauber, and J. A. Mackin. 1966. *Minchinia nelsoni* n. sp. (Haplosporida, Haplosporidiidae): Causative agent of the Delaware Bay oyster epizootic. Science (Wash., D.C.) 153:1414-1416.
- Hewatt, W. G., and J. D. Andrews. 1954. Oyster mortality studies in Virginia. I. Mortalities of oysters in trays at Gloucester Point, York River. Tex. J. Sci. 6:121-133.
- Huggett, R. J., M. E. Bender, and H. D. Slone. 1973. Utilizing metal concentration relationships in the eastern oyster (*Crassostrea virginica*) to detect heavy metal pollution. Water Res. 7:451-460.
- Huggett, R. J., F. A. Cross, and M. E. Bender. In press. Copper and zinc in oysters and sediments from two coastal plain estuaries. In Proceedings of the Symposium on Mineral Cycling in Southeastern Ecosystems, Augusta, Ga. May 1974.
- Mackin, J. G., H. M. Owen, and A. Collier. 1950. Preliminary note on the occurrence of a new protistan parasite, *Dermocystidium marinum* n. sp. in *Crassostrea virginica* (Gmelin). Science (Wash., D.C.) 111:328-329.
- Pauley, G. B. 1969. A critical review of neoplasia and tumorlike lesions in mollusks. In Neoplasms and related disorders of invertebrate and lower vertebrate animals. Natl. Cancer Inst. Monogr. 31:509-539.
- Wolf, P. H. 1969. Neoplastic growth in two Sydney rock oysters, *Crassostrea commercialis* (Iredale and Roughley). In Neoplasms and related disorders of invertebrate and lower vertebrate animals. Natl. Cancer Inst. Monogr. 31:563-573.
- Wood, J. L., and J. D. Andrews. 1962. *Haplosporidium costale* (Sporozoa) associated with a disease of Virginia oysters. Science (Wash., D.C.) 136:710-711.

MFR Paper 1211. From Marine Fisheries Review, Vol. 38, No. 10, October 1976. Copies of this paper, in limited numbers, are available from D825, Technical Information Division, Environmental Science Information Center, NOAA, Washington, DC 20235. Copies of Marine Fisheries Review are available from the Superintendent of Documents, U.S. Government Printing Office, Washington, DC 20402 for \$1.10 each.